Evaluation of the Broiler's Ability to Adapt to an Early Moderate Deficiency of Phosphorus and Calcium

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ABSTRACT We studied the ability of broiler chickens to adapt to early moderate P and Ca deficiencies by evaluating the impact of feeding different concentrations of P and Ca, from 1 to 18 d, on performance, bone characteristics, and nutrient absorption in the grower (Gr) period (18 to 32 d). Two starter (St) diets were fed from 1 to 18 d: a control (C) diet [0.45% nonphytate P (nPP) and 0.9% Ca] and a low (L) diet (0.30% nPP and 0.6% Ca). On d 19, half of the birds fed the St C diet were switched to a Gr C diet (0.40% nPP and 0.8% Ca), and the other half were switched to a Gr L diet (0.30% nPP and 0.6% Ca), whereas those fed the L diet in the St phase were fed the L diet in the Gr phase, resulting in a total of 3 treatments.

Broiler chickens fed the St L diet weighed less (P < 0.05) than those fed the St C diet at 18 d; however, by 23 d they had they caught up to the C-C birds, and no BW differences (P > 0.05) were observed at 28 and 32 d. Feeding the St L diet resulted in decreased (P < 0.05) tibia ash at 18 d, but by 32 d their tibia ash was not different from that of birds fed the St C and Gr L diets. Broilers subjected to P and Ca restriction from hatch to 18 d absorbed more P and Ca during all times sampled than birds fed the St C and Gr C diets or those fed the St C and the Gr L diet. These results demonstrated that modern broilers exhibited a high adaptive capacity when they were exposed to early dietary P or Ca restrictions.

(Key words: broiler, phosphorus, calcium, retention, adaptation)

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INTRODUCTION

Land application of poultry litter, which is comparatively high in P due to limited utilization of phytin P (Nelson, 1976) by poultry, is of increasing concern in areas of intensive poultry production in the United States (Sharpley, 1999). The US Environmental Protection Agency has passed federal regulations that limit the amount of poultry litter that can be applied to soils, based mainly on litter P content (Environmental Protection Agency, 2003). To address the P issue, poultry nutritionists have developed several nutritional strategies that include feeding diets with P concentrations closer to requirements (Yan et al., 2001; Dhandu and Angel, 2003); dietary supplementation with feed additives, such as microbial phytase (Simons et al., 1990; Denbow et al., 1995; Yi et al., 1996) and vitamin D₃ metabolites (Edward, 1993; Biehl and Baker, 1997; Edward, 2002); and use of genetically modified feed ingredients with lower concentrations of phytin P (Cromwell et al., 1998; Li et al., 2000; Waldroup et al., 2000). Most of these strategies, although affective, will affect production costs.

The adaptation of animals to low nutrient diets has been long recognized. Animals respond to nutrient restriction by increasing absorption rates and utilization efficiency, which decreases excretion of the restricted nutrients. The ability of humans to adapt to a diet low in Ca was recognized in the 1950s. At that time, the Food and Nutrition Board (1948) recommended an adult daily Ca allowance of 800 mg/d. However, Hegsted et al. (1952) found that adult Peruvians, who had lived on low Ca diets for long periods, only required 100 to 200 mg Ca/ d to maintain balance. It is obvious that these individuals, who grew up under Ca restriction, were able to better utilize Ca. This theory was tested in a long-term dog feeding trial (Gershoff et al., 1958), in which dogs fed 0.11% Ca in their diet had a Ca retention of 90%, whereas dogs fed diets containing 0.63 and 1.23% Ca had Ca retentions of 46 and 27%, respectively. Dogs fed diets containing 0.11% Ca were able to maintain Ca balance, and chemical and histological examination did not reveal differences in composition or bone abnormalities among the 3 groups of dogs. These authors suggested that adaptation to low Ca diets results in deposition of a more stable

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Abbreviation Key: BMC = bone mineral content; BMD = bone mineral density; C = control; C-C = control-control; C-L = control-low; DXA = dual energy x-ray absorptiometry; Gr = grower; L-L = low-low; nPP = nonphytate P; St = starter; Trt = treatment.

form of Ca and hypothesized that the tendency for older humans and animals to lose Ca may be partly due to a Ca intake that is too liberal during early life.

Adaptation to diets with restricted P and Ca has also been previously reported in chickens. In an in vitro trial, using ligated duodenal loops from broiler chickens, Morrisey and Wasserman (1971) observed a higher percentage of absorption of a labeled ⁴⁷Ca (ranging from 70 to 90%) when diets low in Ca (0.08%) were fed for 8 d prior to intestinal sampling, regardless of dietary P concentrations, or when low P (0.25%) diets were fed, regardless of dietary Ca concentrations. Chickens receiving a diet with normal P (0.65%) and Ca (1.20%) absorb less than 50% of ⁴⁷Ca. Duodenal P absorption in 20-d-old broiler chicks fed a low Ca or a low P diet for 8 d increased by 49 and 87%, respectively (Fox et al., 1981). Blahos et al. (1987) reported an increase in duodenal and ileal P absorption in broiler chickens fed a low Ca diet for 2 wk and a smaller increase in duodenal but not ileal P absorption in chicks fed a low P diet. These adaptations to P or Ca restriction have been hypothesized to be the result of an increased level of circulating 1,25 dihydroxycholecalciferol $[1,25-(OH)_2-D_3]$ (Hunziker et al. 1982; Blahos et al., 1987) and duodenal calbindin content (Morrisey and Wasserman, 1971; Montecuccoli et al., 1977). By comparing the duodenal calbindin concentration and its changing pattern with age for 1991 and 2001 strains of broilers, Bar et al. (2003) concluded that modern broilers exhibit an elevated capacity for adaptation to P or Ca deficiency, and this capacity remains elevated for the whole growth period. However, no literature could be found on work conducted to evaluate the long-term effects of early P or Ca restriction on growth performance, bone mineralization, and P absorption in poultry. This experiment was conducted to evaluate broiler abilities to adapt to a moderate early life deficiency in P and Ca and to characterize the adaptive changes by examining the impact of the previous P and Ca status [starter (St) phase, hatch to 18 d] on performance, bone characteristics, and nutrient absorption of broilers during the grower (Gr) phase (19 to 32 d).

MATERIALS AND METHODS

Diets

Corn and soybean meal mash diets were formulated to meet NRC broiler requirements (NRC, 1994) for all nutrients except Ca and P. For each growth phase (St: 1 to 18 d; Gr: 19 to 32 d), a basal diet was formulated (Table 1) to contain low concentrations of P and Ca (0.47% P and 0.41% Ca). Basal diets were analyzed for P colorimetrically (Heinonen and Lahti, 1981) and Ca by atomic absorption (Perkin Elmer, 1982). Based on analyzed values, appropriate amounts of calcium carbonate, monocalcium

phosphate, and Celite² (used as an indigestible marker and filler at a minimum inclusion of 1%) were added to the basal diet to provide test diets with the desired concentrations of P and Ca. In all test diets the basal diet accounted for 97% of the complete diet. For each growth phase, 2 experimental complete diets were made: a control (C) diet with normal levels of P and Ca [0.45% non-phytate P (nPP) and 0.9% Ca for the St C, 0.40% nPP and 0.8% Ca for the Gr C] and a low (L) diet with reduced concentrations of P and Ca (0.30% nPP and 0.6% Ca for the St and Gr L diets).

Bird Care

All institutional Animal Care and Use Committee guidelines were followed. Day-old male broiler chickens of a commercial strain (Hubbard-Isalic)³ were placed in 77 electronically heated brooder battery pens (99 × 33 × 36 cm, length × width × height, respectively). Test diets and water were provided ad libitum. The following lighting schedules per age were used during the experiment: hatch to 4 d (24L:0D), 5 to 12 d (14L:10D), 13 to 20 d (16L:8D), and 21 to 32 d (20L:4D). Room temperature was maintained at 32.2°C for the first week and was decreased 2.75°C each week thereafter. Mortality was checked twice daily, and the weights of dead birds were used to adjust feed conversion ratios.

Experimental Treatments

The experiment consisted of 3 treatments (Trt): controlcontrol (C-C), control-low (C-L), and low-low (L-L; Table 2). Birds on the C-C Trt were fed the starter and grower control diets, those on the C-L Trt were fed the starter control diet and the grower low diet, and those on the L-L Trt were fed the starter and grower low diets. From 1 to 18 d, all pens of birds assigned to Trt C-C and C-L (49 pens) were fed the St C diet, and those assigned to the L-L Trt (28 pens) were fed the St L diet. On d 18, seven preassigned pens per Trt were sampled, and ileal content, right shank, and tibia were collected. On d 19, birds on all Trt were switched to the Gr diets. Of the 42 remaining pens fed the St C diet, 21 remaining pens were fed Gr C diet (C-C Trt) for the Gr phase, and 21 pens were fed the Gr L (C-L Trt). All 21 pens fed the St L diet in the St phase were fed the Gr L diet in the Gr phase (L-L Trt).

Forty-eight and 96 h after switching to the Gr diets (21 and 23 d of age), and at the end of the trial (32 d of age), 7 pens from each dietary Trt were sampled in an effort to characterize adaptive changes over time. Dietary Trt and sampling date for each Trt were randomly assigned to pens at the beginning of the trial. The pens that were removed on d 18 and 21 had 8 birds per pen, and the pens sampled on d 23 and 32 had 5 birds per pen from the start of the experiment . The choice of different bird numbers was necessary to ensure adequate sample amounts of ileal content for nutrient absorption determinations in the 18 and 21 d samplings as well as adequate

²Seegott Inc. Streetsboro, OH.

³Hubbard, Statesville, NC.

TABLE 1. Composition of the starter (0-18 d) and grower (19-32 d) basal diets

Ingredient	Starter basal	Grower basal
		(g/kg) —
Yellow corn	585.89	627.69
Soybean meal (48%)	342.61	311.25
Soy oil	30.00	35.00
Corn gluten meal	22.22	7.62
Salt	5.92	4.64
Calcium carbonate	4.33	4.50
Monocalcium phosphate	4.04	4.42
DL-Methionine	2.14	2.25
Lysine·HCl	0.75	0.63
Mineral mix ¹	0.70	0.70
Vitamin premix ²	0.70	0.70
Choline chloride 60 %	0.70	0.63
Calculated (analyzed) nutrient concentration		
DM, %	$89.7 (89.6 \pm 0.001)$	$89.6 (89.5 \pm 0.053)$
ME, kcal/kg	3,144	3,198
Crude protein %	22.7	20.6
Ca, %	$0.41 (0.39 \pm 0.004)$	$0.41 \ (0.46 \pm 0.001)$
P, %	$0.47 (0.49 \pm 0.004)$	$0.47 (0.52 \pm 0.005)$
Nonphytate P, ³ %	$0.21 \ (0.21 \pm 0.004)$	$0.21 \ (0.23 \pm 0.005)$

¹Provided per kilogram of diet: zinc (from zinc oxide), 147 ppm; manganese (from manganese sulfate), 84 ppm; iron (from ferrous sulfate), 28 mg; copper (from copper oxide), 14 mg; iodine (from calcium iodate), 2.1 ppm; cobalt (from cobalt carbonate), 0.035 mg.

 2 Provided per kilogram of diet: vitamin A (from retinyl acetate), 13,135 IU; cholecalciferol, 4,636 ICU; vitamin E (from DL-α-tocopheryl acetate), 46.4 IU; vitamin B₁₂ (from cyanocobalamin), 0.023 mg; riboflavin (from riboflavin), 15.5 mg; niacin (from nicotinic acid), 61.8 mg; pantothenic acid (from calcium D-pantothenate), 21.6 mg; vitamin K3 (from menadion sodium bisulfite complex), 2.78 mg; folic acid (from folic acid), 1.85 mg; pyridoxine (from pyridoxine hydrochloride), 5.41 mg; thiamin (thiamine mononitrate) 3.86 mg; and (from D-biotin), 0.124 mg.

³Determined by subtracting analyzed phytate P from analyzed P.

space to maximize growth in the birds sampled at 23 and 32 d.

Live Bird Measurement, Sampling, and Laboratory Measurements

At each sampling time, BW and feed consumption was determined on a pen basis. Ileal digestive contents (from Meckel's diverticulum to 2 cm above the ileocecal junction) were collected and pooled by pen. Ileal samples were freeze-dried, ground (0.5-mm screen), and stored at 10°C for further analysis. Right tibias were removed and defleshed (cartilage cap removed), and right shanks were removed. Bones were pooled by bone type and pen and frozen (–4°C) until analyzed. Bone mineral content (BMC) and bone mineral density (BMD) were measured on the thawed bones by using dual energy x-ray absorptiometry (DXA;⁴ Mitchell et al., 1997). The bones (grouped by pen bone type) were placed on a 7 mm thick Lucite tablet and scanned using the Small Animal Total Body software⁵ in the high resolution-medium scan mode. Results were expressed as the mean tibia or shank BMC (g/bone) and mean BMD (g/cm²) per pen. Tibia ash was determined on a dry defatted basis (AOAC, 1990).

Body weights were also recorded on d 8, 14, and 28 in an effort to monitor how growth rate responded to dietary

P and Ca status throughout the test period. Feed consumption was determined at the same times as BW. All test diets and dried ileal contents were ground to pass through a 0.50-mm screen and then analyzed in duplicate for dry matter (AOAC, 1990), P, Ca, and PP (Rounds and Nielsen, 1993) as modified by Newkirk and Classen (1998) and acid-insoluble ash (Vogtmann et al., 1975). The nPP concentrations for the diets were determined by subtracting analyzed PP from analyzed P. Apparent ileal absorption of P and Ca and disappearance of PP were calculated using a marker method based on acid-insoluble ash analysis in which Celite was used as an indigestible marker (Scott and Boldaji, 1997).

Statistical Data Analyses

A pen of birds was the experimental unit for all measurements. Data were subjected to one-way ANOVA using the GLM procedure of SAS software (JMP, 2000). Mortality data were transformed into square root of n + 1 for statistical analysis, but the data are presented as natural numbers. When the overall model was significant ($P \ge 0.05$), means were separated by Tukey's honestly significant difference test (Tukey, 1991). Probability was considered significant when $P \le 0.05$.

From 19 to 23 d, three pens from the C-L dietary Trt group lost one bird each. Upon a one-way ANOVA test within this Trt group, the pens that had lost a bird were found to have better (P < 0.05) performance at 32 d than the other 4 pens in that Trt. This effect was apparently

⁴LunarTM DPX-L, Lunar Corp., Madison, WI 53700.

⁵Version 4.6d, Lunar Corp., Madison, WI.

TABLE 2. Formulated and determined nonphytate P1 (nPP) and Ca

	nl	PP		Ca
Diet	Formulated	Determined	Formulated	Determined
Starter control Starter low Grower control Grower low	0.45 0.30 0.40 0.30	0.43 ± 0.03 0.29 ± 0.01 0.40 ± 0.01 0.29 ± 0.00	0.90 0.60 0.80 0.60	0.95 ± 0.01 0.63 ± 0.02 0.86 ± 0.01 0.65 ± 0.03

¹The nonphytate P values were determined by subtracting analyzed phytate P from analyzed P.

related to a limitation in feeder space when there where 5 vs. 4 birds per pen between 23 and 32 d of age. The data from the 3 pens that lost one bird were, therefore, removed from the statistical analysis. For consistency of approach, one pen from the C-C dietary Trt group was also removed for the same reason.

RESULTS

Determined Diet Nutrient Concentrations

The formulated and determined nPP and Ca levels for test diets are shown in Table 2. There was good agreement between formulated and analyzed levels.

Nonphytate P and Ca Consumption

As expected, nPP and Ca consumption were directly related to dietary P and Ca concentrations (Tables 3 and 4). Broiler chickens fed the St C diet consumed more nPP and Ca from 1 to 8, 8 to 14, and 14 to 19 d than birds fed the St L diet. From 19 to 21, 21 to 23, 23 to 28, and 28 to 32 d of age, nPP and Ca consumptions of birds fed on the C-C Trt were higher than those of C-L and L-L Trt birds, and there was no difference between these last 2 groups. Cumulative nPP or Ca consumption (1 to 21, 1 to 23, 1 to 28, and 1 to 32 d) was always highest for the C-C Trt group, followed by the C-L Trt group, and the lowest for the L-L Trt group.

Performance

The effects of dietary Trt on BW of broiler chickens at different ages are shown in Table 5. There were no Trt effects on BW at 8 d of age. At 14 and 19 d of age, broiler chickens fed the St C diet were heavier than those fed the St L diet. On d 21, broiler chickens fed C-C and C-L treatment diets had similar BW but were heavier than those fed the L-L Trt diets. On d 23, broiler chickens fed the L-L Trt diets were no longer different in BW from those fed the C-C Trt diets; however, those fed the C-L Trt diets were unexplainably heavier. At d 28 and 32, no differences were observed among the 3 groups.

The effects of Trt on feed consumption are shown in Table 6. Broiler chickens fed the St L diet consumed less feed than birds fed the St C diet from d 8 to 14 and d 14 to 19 of age. No effect of Trt on feed consumption was found from d 1 to 8, 19 to 21, 21 to 23, 23 to 28, or 28 to

TABLE 3. Nonphytate phosphorus (nPP) consumption of broiler chickens for different age periods as affected by dietary treatment^{1,2}

	nPF	consumed (g	5)		
Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
1-8 1-14 1-19 1-21 1-23 1-28	1.5	54 ^a 52 ^a 37 ^a 3.35 ^b 3.90 ^b 5.48 ^b	0.37 ^b 1.00 ^b 1.86 ^b 2.32 ^c 2.80 ^c 4.35 ^c	0.006 0.011 0.021 0.033 0.041 0.075	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001 <0.0001
1-32 8-14 14-19 19-21	8.04 ^a 0.9 1.3	6.75 ^b 98 ^a 35 ^a 0.47 ^b	5.66 ^c 0.64 ^b 0.87 ^b 0.46 ^b	0.074 0.007 0.010 0.009	<0.0001 <0.0001 <0.0001 <0.0001
21-23 23-28 28-32	0.71 ^a 2.16 ^a 1.80 ^a	0.56 ^b 1.60 ^b 1.27 ^b	0.51 ^b 1.55 ^b 1.31 ^b	0.017 0.041 0.023	<0.0001 <0.0001 <0.0001

 $^{\text{a-c}}$ Means within rows with common superscripts do not differ (P < 0.05).

¹Up to d 14, data are means of 49 and 28 pens for broiler chickens on treatments fed the starter (St) control (C) and St low (L) diets, respectively. For d 19, data are means of 42 and 21 pens for broiler chickens fed St C and L diets, respectively. For d 21, data are means of 21 pens. For d 23, data are means of 13, 11, and 14 pens for broiler chickens on the C-C, C-L, and L-L treatment, respectively. For d 28 and 32, data are means of 6, 4, and 7 pens for broiler chickens C-C, C-L, and L-L treatment, respectively.

²Determined nPP concentrations were 0.43 and 0.29% for the St C and L diets, respectively, and 0.40 and 0.29% for the grower (Gr) C and L diets, respectively. The analyzed Ca concentrations were 0.95 and 0.63% for the St C and L diets, respectively, and 0.86 and 0.65% for the Gr C and L diets, respectively.

³Standard error of means (weighted when n was not equal).

32. Overall feed consumption (hatch to 32 d) was not affected by Trt.

Feed to gain ratio was affected by Trt from 1 to 8, and 8 to 14 d (Table 7); broiler chickens fed the St C diet converted feed into gain more efficiently than those on St L diet. However, after d 19, the overall feed efficiency was not different among Trt. There was no effect of Trt on mortality (Table 8) for any period studied.

Bone Characteristics

Tibia and shank BMD and BMC measured by DXA are shown in Table 9. At 18 d, tibia BMC was reduced when the St L diet was fed. At d 21, 23, and 32, the broiler chickens on the C-C Trt had the highest BMC, whereas those on the L-L Trt had the lowest. Feeding broiler chickens diets low in P and Ca from 1 to 18 d of age also resulted in a lowered tibia BMD at 18 d, and BMD remained low at all other sampling times. When birds fed the St C diet were switched to the L diet in the Gr phase, there was no effect on tibia BMD. Shank BMC followed the same trend as tibia BMD. Shank BMD at 18 and 21 d was also lower in birds fed the St L diet. However, at 23 and 32 d, there was no difference in shank BMD among the 3 Trt groups.

Dry-defatted tibia weight, tibia ash weight, and tibia ash percentage responded to dietary Trt in a similar manner as that observed for tibia BMD and BMC (Table 10).

TABLE 4. Calcium consumption by broiler chickens for different age periods as affected by dietary treatment^{1,2}

	Ca	consumed (g)		
Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
1-8 1-14 1-19	3.3	1.19 ^a 3.36 ^a 6.34 ^a		0.013 0.024 0.046	<0.0001 <0.0001 <0.0001
1-21 1-23 1-28 1-32	7.72 ^a 9.10 ^a 13.58 ^a 17.44 ^a	7.42 ^b 8.65 ^b 12.18 ^b 15.03 ^b	4.04 ^b 5.07 ^c 6.15 ^c 9.63 ^c 12.56 ^c	0.072 0.090 0.165 0.163	<0.0001 <0.0001 <0.0001 <0.0001
8-14 14-19 19-21 21-23 23-28	2.2		1.37 ^b 1.88 ^b 1.04 ^b 1.15 ^b 3.48 ^b 2.94 ^b	0.016 0.022 0.019 0.036 0.089 0.051	<0.0001 <0.0001 <0.0001 <0.0001 <0.0001

 $^{^{\}text{a-c}}$ Means within rows with common superscripts do not differ (P < 0.05).

¹Up to d 14, data are means of 49 and 28 pens for broiler chickens on treatments fed the starter (St) control (C) and St low (L) diets, respectively. For d 19, data are means of 42 and 21 pens for broiler chickens fed St C and L diets, respectively. For d 21, data are means of 21 pens. For d 23, data are means of 13, 11, and 14 pens for broiler chickens on the C-C, C-L, and L-L treatment, respectively. For d 28 and 32, data are means of 6, 4, and 7 pens for broiler chickens C-C, C-L, and L-L treatment, respectively.

²Determined nonphytate P concentrations were 0.43 and 0.29% for the St C and L diets, respectively, and 0.40 and 0.29% for the grower (Gr) C and L diets, respectively. The analyzed Ca concentrations were 0.95 and 0.63% for the St C and L diets, respectively, and 0.86 and 0.65% for the Gr C and L diets, respectively.

³Standard error of means (weighted when n was not equal).

At 18 d, the 3 measurements were reduced in broiler chickens fed the St L diet as compared with those fed the St C diet. For broilers fed the St C diet, switching to the Gr L diet lowered tibia weight and tibia ash weight at d 21 and 32 d of age and tibia ash percentage at d 21, 23, and 32 d of age. All 3 measurements were lower for birds in the L-L Trt than for those in the other 2 Trt groups at 21 and 23 d. However, by 32 d of age birds in this Trt had tibia dry-defatted weight, tibia ash weight, and tibia ash percentage were similar to those of broiler chickens fed the C-L Trt diets but remained lower than those of birds fed the C-C Trt diets. When tibia ash weight was expressed as grams of nPP or Ca consumed, per gram of ash, broiler chickens fed the L-L Trt diets had higher ash weight per gram of nPP or Ca consumed at all ages as compared with that of birds fed the C-C or C-L Trt diets, and no difference was observed between these last 2 Trt.

Nutrient Absorption

At d 18, the apparent ileal absorption of P for broiler chickens fed the St L diet was 56.0%, which was relatively 13% higher than that for birds fed the St C diet (Table 11). On d 21 and 23, chicks fed the L-L Trt diets absorbed 26 and 36% relatively more P than those fed the C-L Trt diets, and there was no difference between C-C and C-L Trt groups. On d 32 of age, apparent ileal absorption of P in broiler chickens fed L-L Trt diets decreased dramati-

TABLE 5. Body weight and body weight gain of broiler chickens at different ages as affected by dietary treatment^{1,2}

Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
		— (g) —			
Body weight		(0)			
1		38	38	0.1	0.5300
8	1	39	136	1.2	0.1338
14	3	15 ^a	299 ^b	2.7	< 0.0001
19	5	39 ^a	508^{b}	4.9	< 0.0001
21	650^{a}	650 ^a	621 ^b	7.1	0.0080
23	$740^{\rm b}$	774^{a}	722^{b}	8.7	0.0010
28	1,065	1,131	1,068	18.2	0.0621
32	1,346	1,373	1,338	18.2	0.4457
Body weight gain					
1-8	1	01	98	1.3	0.1242
8-14	1:	76 ^a	163 ^b	1.7	< 0.0001
14-19	2:	24 ^a	213^{b}	2.5	0.0045
19-21	111	106	113	3.0	0.2674
21-23	106 ^b	125 ^a	115 ^{ab}	5.0	0.0381
23-28	334	357	346	13.5	0.5624
28-32	282	243	270	12.2	0.1384
1-32	1,308	1,335	1,300	16.8	0.4576
19-32	807	834	830	14.5	0.2897

 a,b Means within rows with common superscripts do not differ (P < 0.05).

¹Up to d 14, data are means of 49 and 28 pens for broiler chickens on treatments fed the starter (St) control (C) and St low (L) diets, respectively. For d 19, data are means of 42 and 21 pens for broiler chickens fed St C and L diets, respectively. For d 21, data are means of 21 pens. For d 23, data are means of 13, 11, and 14 pens for broiler chickens on the C-C, C-L, and L-L treatment, respectively. For d 28 and 32, data are means of 6, 4, and 7 pens for broiler chickens C-C, C-L, and L-L treatment, respectively.

²Determined non-phytate phosphorus concentrations were 0.43 and 0.29% for the St C and L diets, respectively, and 0.40 and 0.29% for the grower (Gr) C and L diets, respectively. The analyzed Ca concentrations were 0.95 and 0.63% for the St C and L diets, respectively, and 0.86 and 0.65% for the Gr C and L diets, respectively.

³Standard error of means (weighted when n was not equal).

cally to 47.2% from 57.1% at 23 d of age, which was still higher than that of birds fed the C-C Trt diet but no longer differed from that for birds fed the C-L Trt diets.

On d 18 of age, birds fed the St L diet hydrolyzed 3 times more PP than birds fed the St C diet (37.2 and 12.3% PP hydrolysis, respectively). Apparent PP hydrolysis at 21 and 23 d of age in broiler chickens fed the L-L Trt diets was higher than that in the C-L Trt fed birds. But apparent PP hydrolysis at d 21 and 23 in C-L Trt fed birds was higher than that in birds fed the C-C Trt diets. By d 32, no differences were observed among any of the Trt.

On d 18 of age, broiler chickens fed the St L diet absorbed more Ca than those fed the St C diet (Table 11). At 21 and 23 d of age, Ca absorption in birds fed the C-L and C-C Trt diets was lower than that in birds fed the L-L Trt diets, and there was no difference between birds on these 2 last Trt. On d 32 of age, Ca absorption by birds fed the L-L Trt diets decreased to 60.5% from 72.0% at 23 d of age but was still higher than that of birds fed the C-C Trt diets (48.2%) but no longer different from that of birds fed the C-L Trt diets (57.7%).

DISCUSSION

The fact that broiler chickens on the L-L Trt caught up with those on the C-C Trt in terms of BW at 23 d and

TABLE 6. Feed consumption of broiler chickens for different age periods as affected by dietary treatment^{1,2}

Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
		— (g) ——			
1-8	1	25	126	1.4	0.7393
1-14	3	54	346	2.7	0.0526
1-19	68	19 ^a	641 ^b	5.1	0.0012
1-21	827 ^{ab}	832 ^a	800^{b}	8.4	0.0218
1-23	990 ^{ab}	1,021 ^a	966 ^b	10.8	0.0049
1-28	1,511	1,560	1,501	21.4	0.1978
1-32	1,960	1,999	1,953	22.4	0.3978
8-14	2	29 ^a	220 ^b	1.7	0.0015
14-19	3	14 ^a	298 ^b	2.5	< 0.0001
19-21	159	161	160	2.7	0.8788
21-23	177	192	177	5.1	0.0906
23-28	540	552	535	11.5	0.6487
28-32	450	439	452	7.4	0.4863

 $^{^{\}mathrm{a,b}}\mathrm{Means}$ within rows with common superscripts do not differ (P < 0.05).

¹Up to d 14, data are means of 49 and 28 pens for broiler chickens on treatments fed the starter (St) control (C) and St low (L) diets, respectively. For d 19, data are means of 42 and 21 pens for broiler chickens fed St C and L diets, respectively. For d 21, data are means of 21 pens. For d 23, data are means of 13, 11, and 14 pens for broiler chickens on the C-C, C-L, and L-L treatment, respectively. For d 28 and 32, data are means of 6, 4, and 7 pens for broiler chickens C-C, C-L, and L-L treatment, respectively.

²Determined non-phytate phosphorus concentrations were 0.43 and 0.29% for the St C and L diets, respectively, and 0.40 and 0.29% for the grower (Gr) C and L diets, respectively. The analyzed Ca concentrations were 0.95 and 0.63% for the St C and L diets, respectively, and 0.86 and 0.65% for the Gr C and L diets, respectively.

³Standard error of means (weighted when n was not equal).

TABLE 7. Feed conversion ratio of broiler chickens for different age periods as affected by dietary treatment^{1,2}

Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
		(g)			
1.0	1.25 ^b	_	1 200	0.012	0.0240
1-8		1.25 ^a	1.29 ^a	0.013	0.0249
1-14	1.28 ^b	1.28 ^b	1.33 ^a	0.006	< 0.0001
1-19	1.34^{b}	1.34^{b}	1.37^{a}	0.005	0.0013
1-21	1.35	1.36	1.38	0.007	0.0618
1-23	1.41	1.39	1.41	0.011	0.2388
1-28	1.47	1.43	1.460	0.014	0.1574
1-32	1.50	1.50	1.50	0.011	0.9075
8-14	1.30 ^b		1.36 ^a	0.007	< 0.0001
14-19	1.40		1.40	0.009	0.9851
19-21	1.44 ^{ab}	1.51 ^b	1.44 ^a	0.019	0.0270
21-23	1.58	1.54	1.54	0.027	0.5116
23-28	1.63	1.55	1.55	0.036	0.2390
28-32	1.70	1.76	1.68	0.033	0.3099

 $^{^{}a,b}$ Means within rows with common superscripts do not differ (P < 0.05).

¹Up to d 14, data are means of 49 and 28 pens for broiler chickens on treatments fed the starter (St) control (C) and St low (L) diets, respectively. For d 19, data are means of 42 and 21 pens for broiler chickens fed St C and L diets, respectively. For d 21, data are means of 21 pens. For d 23, data are means of 13, 11, and 14 pens for broiler chickens on the C-C, C-L, and L-L treatment, respectively. For d 28 and 32, data are means of 6, 4, and 7 pens for broiler chickens C-C, C-L, and L-L treatment, respectively.

²Determined nonphytate phosphorus concentrations were 0.43 and 0.29% for the St C and L diets, respectively, and 0.40 and 0.29% for the grower (Gr) C and L diets, respectively. The analyzed Ca concentrations were 0.95 and 0.63% for the St C and L diets, respectively, and 0.86 and 0.65% for the Gr C and L diets, respectively.

³Standard error of means (weighted when n was not equal).

TABLE 8. Mortality of broiler chickens for different age periods as affected by dietary treatment^{1,2}

Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
		— (%) ——			
1-8	0.	76	0.00	0.46	0.1864
8-14	0.	66	1.61	0.70	0.3173
14-19	0	0	0		
19-21	0	0.95	0	0.48	0.0898
21-23	1.42	2.85	0	1.84	0.5583
23-28	0	0	0		
28-32	5.71	0	5.71	3.93	0.5068

¹Up to d 14, data are means of 49 and 28 pens for broiler chickens on treatments fed the starter (St) control (C) and St low (L) diets, respectively. For d 19, data are means of 42 and 21 pens for broiler chickens fed St C and L diets, respectively. For d 21, data are means of 21 pens. For d 23, data are means of 13, 11, and 14 pens for broiler chickens on the C-C, C-L, and L-L treatment, respectively. For d 28 and 32, data are means of 6, 4, and 7 pens for broiler chickens C-C, C-L, and L-L treatment, respectively.

²Determined nonphytate phosphorus concentrations were 0.43 and 0.29% for the St C and L diets, respectively, and 0.40 and 0.29% for the grower (Gr) C and L diets, respectively. The analyzed Ca concentrations were 0.95 and 0.63% for the St C and L diets, respectively, and 0.86 and 0.65% for the Gr C and L diets, respectively.

³Standard error of means (weighted when n was not equal).

continued to be similar until the end of the trial could be explained by 2 reasons. First, broilers fed the diet with low P and Ca demonstrated a certain ability to adapt to this moderate deficiency, which was further demonstrated by the increased total P and Ca apparent absorption and PP hydrolysis and will be further discussed later. Second, although the Gr L diet and the St L diet were formulated to have the same levels of P and Ca and analyzed to contain very similar levels of P and Ca, the most rapid growth of bones occurs during the first 3 wk, and a bird's requirement of P and Ca decreases with age; therefore, the restriction was less severe in the Gr phase than in the St phase. This result allowed for similar performance for birds on the C-L diet and C-C birds. Birds fed adequate P during the St phase can tolerate relatively low levels of P later on without limiting growth rate. Angel et al. (2000) reported that broilers from 18 to 32 d of age that are fed a St diet meeting NRC (1994) nutrient recommendations, nPP has a quadratic effect on BW gain, and the inflection point is between 0.28 and 0.32% nPP. Yan et al. (2001) found that broilers grown on a diet adequate in P (0.45% nPP) and Ca (1.0%) up to 3 wk only required 0.186% nPP from 3 to 6 wk of age for maximum BW gain. In the current study, the Gr low diet was analyzed to contain 0.30% nPP, and it did not have a negative effect on BW in broilers fed the St C diet, which agreed with the findings of Angel et al. (2000) and Yan et al. (2001). It is expected that the dietary Trt used in this study would not affect mortality because the nPP requirements for minimum mortality are lower than the nPP requirement for maximum BW or optimum feed conversion ratio (Waldroup et al., 2000).

Tibia ash percentage has been the traditional criterion to measure the degree of bone mineralization in broilers. Broiler chickens that consumed St L diet from hatch to

TABLE 9. Tibia and shank bone mineral density (BMD) and bone mineral content (BMC) of 18-, 21-, 23-, and 32-d-old broilers as affected by dietary treatment 1,2

Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
Tibia BMC ⁴ (g)					
18	0.123^{a}		0.044^{b}	0.003	< 0.0001
21	0.269^{a}	0.215^{b}	0.128^{c}	0.008	< 0.0001
23	0.313^{a}	0.263 ^b	0.163^{c}	0.015	< 0.0001
32	0.794^{a}	0.618^{b}	0.522^{c}	0.034	< 0.0001
Tibia BMD ⁵ (g/cm ²)					
18	0.195^{a}		0.178^{b}	0.001	< 0.0001
21	0.211a	0.209^{a}	0.192^{b}	0.001	< 0.0001
23	0.211a	0.210^{a}	0.198^{b}	0.001	< 0.0001
32	0.233a	0.226^{a}	0.220^{b}	0.002	0.0085
Shank BMC (g)					
18	0.042^{a}		0.016^{b}	0.002	< 0.0001
21	0.090^{a}	0.076^{a}	0.035^{b}	0.006	< 0.0001
23	0.106^{a}	0.084^{a}	0.046^{b}	0.008	0.0002
32	0.274^{a}	0.202^{a}	0.162^{b}	0.022	0.0058
Shank BMD (g/cm ²)					
18	0.190^{a}		0.183^{b}	0.001	0.0076
21	0.199^{a}	0.201^{a}	0.186^{b}	0.002	0.0001
23	0.200	0.196	0.192	0.002	0.0589
32	0.219	0.210	0.209	0.003	0.0999

 $^{^{\}mathrm{a,b}}$ Means within rows with common superscripts do not differ (P < 0.05).

18 d had 46.5% tibia ash, 8.5% less than that of broilers fed the St C diet during the same period. After switching to the Gr diets, broiler chickens on the C-C and C-L Trt maintained their tibia ash percentage, whereas those on the L-L Trt were able to increase their tibia ash (49.7% at 32 d vs. 46.5% at 18 d), although they were fed the same diet as birds on the C-L Trt. Even though broiler chickens on the L-L Trt were able to catch up in tibia ash percentage with that of birds on the C-L Trt, they still had lower tibia ash percentages than broilers on the C-C Trt. When tibia ash is considered in terms of consumption (per g) of nPP and Ca, it is clear that broilers fed the St diet low in P and Ca from hatch deposited P and Ca more efficiently than those fed the C diets throughout the experiment as well as those that were switched from the St C diet to the Gr L diet, which could be attributed to an adaptation to the P and Ca restricted diets in the L-L Trt birds.

The BMC and BMD measured by DXA appeared to be good indicators of P and Ca nutrition, with BMC being more sensitive than BMD, and measurements on the tibia seemed to be more sensitive than those on the shanks. BMD measures the projection density (g/cm²) of bones and does not necessarily reflect bone size, whereas BMC is a function of density and size. Birds restricted at an early age can compensate in BMD. They might, however,

have limited ability to compensate in bone size; therefore in this experiment, tibia BMC appeared to be even more sensitive than tibia ash percentage. However, higher BMC only indicates more total bone mineral content and does not necessarily mean stronger bones, because it can be a result of relatively larger bones.

The NRC (1994) P and Ca recommendations are mainly based on maximizing bone ash. Waldroup et al. (2000) reported that as dietary P concentration increased from deficient to excessive, excreta P concentrations increased gradually until the point at which tibia ash percentage was at a maximum and then increased steeply. With the increasing concern in the potential pollution of excreta P, it is essential not to overfeed broilers with P. When we consider that breed, nutrition, and management can all affect growth rate and, therefore, a bird's P and Ca requirements, it becomes an extremely difficult issue to formulate poultry diets to maximize bone ash and, at the same time, avoid excessive P excretion, which increases rapidly once bone ash is maximized. Therefore, the question that needs to be answered is whether it is necessary to feed broiler chickens to maximum bone ash. It is important to feed broiler chickens P concentrations that minimize carcass downgrades and carcass losses in the processing plants due to bone breakage or bone weakness; however, it is not clear whether maximum bone ash is required for minimum processing plant downgrades.

Broiler chickens fed the St L diet exhibited a higher ability to absorb P (49.5 vs. 56.0%) and Ca (60.9 vs. 71.1%) at 18 d than those fed the St C diet. The increased nutrient absorptive ability was maintained at 21 and 23 d and decreased slightly at 32 d but was still higher than that of the broiler chickens fed the C-C Trt diets, which clearly demonstrated that broilers exposed to a moderate deficiency of P and Ca, in the St phase, were able to adapt to these deficiencies. Blahos et al. (1987) also found that chicks fed a diet deficient in Ca for 2 wk showed an increase in duodenal and ileal absorption of P (labeled with ³²P), and when a diet deficient in P was fed for 2 wk, a smaller but still significant increase was found in duodenal but not ileal phosphate absorption. They suggested that the adaptation phenomenon was due in part to a compensatory increase in 1,25-dihydroxy-vitamin D₃ in both deficient diets. Morrisey and Wasserman (1971) fed 9 groups of 17-d-old chicks diets with different concentrations of P and Ca in a 3 × 3 factorial arrangement (0.25, 0.65, and 1.20% P and 0.08, 1.20, and 2.32% Ca) for 10 d and found that duodenal absorption of Ca was increased by lower dietary concentrations of P or Ca alone, regardless of the concentration of the other. Fox et al. (1981) also observed stimulation in the duodenal absorption of phosphate by a low P or Ca diet in chicks. Ravindran et al. (2000), however, found that decreasing nPP from 0.45 to 0.23% did not improve ileal P absorption under each of the 3 phytic acid levels tested (10.4, 13.2, and 15.7%) in broilers fed the Trt diets for 21 d when phytase was not present.

Several possible mechanisms might be involved in an animal's adaptation to P and Ca deficiency. Calbindin is

¹For d 18 and 21, data are means of 7 pens with 8 birds per pen. For d 23 and 32, data are means of 6, 4, and 7 pens with 5 birds per pen for control-control, control-low, and low-low groups, respectively.

 $^{^2\}mathrm{Determined}$ nonphytate P levels were 0.43% and 0.2% for the control and low starter diets respectively, 0.40 and 0.29% for the control and low grower diets, respectively. The analyzed Ca levels were 0.95 and 0.63% for the control and low starter diets, respectively, and 0.86 and 0.65% for the control and low grower diets, respectively.

³Standard error of mean (weighted when n was not equal).

⁴Bone mineral content (BMC).

⁵Bone mineral density (BMD).

TABLE 10. Weight, ash weight, and ash percentage of left tibia for 18-, 21-, 23-, and 32-d-old broilers as affected by dietary treatments^{1,2}

Age (d)	Control- control	Control- low	Low- low	SEM ³	<i>P</i> -value
Tibia weight (g)					
18	1.002 ^a		$0.875^{\rm b}$	0.016	< 0.0001
21	1.460^{a}	1.318 ^b	1.227 ^c	0.029	< 0.0001
23	1.640^{a}	1.585 ^a	$1.402^{\rm b}$	0.028	< 0.0001
32	3.037^{a}	2.796 ^b	2.735 ^b	0.060	0.0008
Tibia ash					
weight (g)					
18	0.509^{a}		0.407^{b}	0.008	< 0.0001
21	0.748^{a}	0.663^{b}	0.584^{c}	0.014	< 0.0001
23	0.838^{a}	0.793^{a}	$0.672^{\rm b}$	0.015	< 0.0001
32	1.588a	1.408^{b}	1.356 ^b	0.034	< 0.0001
Ash weight:					
nonphytate P consumption (g:g)					
18	0.199^{b}		0.240^{a}	0.004	< 0.0001
21	$0.204^{\rm b}$	0.196^{b}	0.245^{a}	0.003	< 0.0001
23	0.199^{b}	0.203^{b}	0.240^{a}	0.004	< 0.0001
32	0.200^{b}	0.209^{b}	0.242^{a}	0.004	< 0.0001
Ash weight:Ca					
consumption (g:g)					
18	0.090^{b}		0.111 ^a	0.002	< 0.0001
21	0.093^{b}	0.089^{b}	0.112a	0.001	< 0.0001
23	0.091^{b}	0.091 ^b	0.109^{a}	0.002	< 0.0001
32	0.092^{b}	0.094^{b}	0.109^{a}	0.002	< 0.0001
Tibia ash (%)					
18	50.8 ^a		46.5^{b}	0.2	< 0.0001
21	51.3 ^a	50.4^{b}	47.6°	0.2	< 0.0001
23	51.1ª	50.0 ^b	47.9°	0.2	< 0.0001
32	52.3ª	50.3 ^b	49.7 ^b	0.3	< 0.0001

^{a-c}Means within rows with common superscripts do not differ significantly (P < 0.05).

TABLE 11. Total P apparent absorption, phytate P (PP) disappearance, and Ca apparent absorption up to distal ileum of 18-, 21-, 23-, and 32-d-old broilers as affected by dietary treatment^{1,2}

Age (d)	Control-control	Control-low	Low-low	SEM ³	<i>P</i> -value
		(%)			
Total P apparent absorption					
18	49.5 ^b		56.0 ^a	1.3	0.0034
21	44.1^{b}	43.5 ^b	54.7 ^a	1.0	< 0.0001
23	43.5 ^b	42.8 ^b	57.1a	2.2	0.0006
32	$41.7^{\rm b}$	44.7^{ab}	47.2^{a}	1.4	0.0445
PP disappearance					
18	12.3 ^b		37.2a	2.1	< 0.0001
21	7.3 ^c	21.1 ^b	35.3a	2.3	< 0.0002
23	6.8 ^c	18.5 ^b	40.0^{a}	3.2	< 0.0001
32	6.0	12.6	15.5	4.5	0.1836
Ca apparent absorption					
18	60.9 ^b		71.1 ^a	2.0	0.0033
21	55.5 ^b	58.5 ^b	70.7^{a}	0.9	< 0.0001
23	57.7 ^b	59.2 ^b	72.0^{a}	2.2	0.0003
32	48.2°	57.6 ^{ab}	60.5 ^a	3.9	0.0323

^{a-c}Means in rows with common superscripts do not differ significantly (P < 0.05).

¹For d 18 and 21, data are means of 7 pens with 8 birds per pen. For d 23 and 32, data are means of 6, 4, and 7 pens with 5 birds per pen for control-control, control-low, and low-low groups, respectively.

²Determined nonphytate P levels were 0.43 and 0.29% for the control and low starter diets respectively, and 0.40 and 0.29% for the control and low grower diets, respectively. The analyzed Ca levels were 0.95 and 0.63% for the control and low starter diets, respectively, and 0.86 and 0.65% for the control and low grower diets, respectively.

³Standard error of mean (weighted when n was not equal).

¹For d 18 and 21, data are means of 7 pens with 8 birds per pen. For d 23 and 32, data are means of 6, 4, and 7 pens with 5 birds per pen for control-control, control-low, and low-low groups, respectively.

²Determined non-PP levels were 0.43 and 0.29% for the control and low starter diets respectively, and 0.40 and 0.29% for the control and low grower diets, respectively. The analyzed Ca levels were 0.95 and 0.63% for the control and low starter diets, respectively, and 0.86 and 0.65% for the control and low grower diets, respectively.

³Standard error of mean (weighted when n was not equal).

the first molecule that is found to change directly with changes in Ca absorption during the adaptation process and has a correlation coefficient of 0.99 between Ca absorption and calbindin (Morrisey and Wasserman, 1971). Calbindin is also considered as a reliable indicator of adaptation (Bar et al, 1990). A 2- to 3-fold increase in duodenal plasma membrane Ca pump mRNA was observed in chicks adapted to a Ca or P deficient diet (Cai et al., 1993). With the successful molecular cloning of a small intestine phosphate cotransporter in mice (Hilfiker et al., 1998) and humans (Xu et al., 2002), studies have been carried out to test its role in the adaptation to P restriction. It has been shown in mice (Hattenhauer et al., 1999) and goats (Huber et al., 2002) that this transporter played an important role in the adaptation of Pi transport to a low P diet, mediated through a post-transcriptional process. In chickens, no work has been done to study the role of the small intestinal phosphate cotransporter in the adaptation process, and it is possible that it might also play a role. Therefore, the adaptation process might involve increased production of calbindin, Ca pump, and intestinal phosphate cotransporter; however, the factor that initiates all these processes is still unknown. Early in the 1950s, Nicolaysen et al. (1953) suggested that the degree of bone mineralization was a primary determinant of Ca absorption in the adaptation process and undermineralization sent a signal to stimulate intestinal Ca absorption. In the literature, the initiating signal was most probably 1,25-dihydroxy vitamin D₃, which has been shown to be elevated in the adaptation process (Bar et al., 1982; Hunziker et al., 1982).

In summary, broilers fed a diet moderately deficient in P and Ca from hatch to 18 d demonstrated the ability to partially adapt to the deficiency, which was shown by the increased ileal absorption of P and Ca, the increased ileal PP disappearance, compensatory growth, and compensatory improvement in bone parameters, including tibia ash, tibia, and shank BMD and BMC in a later growth phase (18 to 32 d). However, practical application of this adaptation process requires more studies to further finetune the degree and period of the restriction with the aim of achieving comparative bone parameters and performance as those of broilers fed diets that meet requirements throughout the growth phase. The application of the adaptation principle in poultry may allow for decreasing fed and excreted P and Ca without limiting performance and provide an additional low cost tool to decrease P and Ca in poultry litter.

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